

WHAT IS CLAIMED IS:

~~1.~~ A method of generating a herpesvirus amplicon particle, the method comprising

providing a cell that has been stably transfected with a nucleic acid sequence that encodes an accessory protein; and

transfecting the cell with (a) one or more packaging vectors that, individually or collectively, encode one or more HSV structural proteins but do not encode a functional herpesvirus cleavage/packaging site and (b) an amplicon plasmid comprising a sequence that encodes a functional herpesvirus cleavage/packaging site and a herpesvirus origin of DNA replication.

~~2.~~ A method of generating a herpesvirus amplicon particle, the method comprising transfecting a cell with

(a) one or more packaging vectors that, individually or collectively, encode one or more HSV structural proteins but do not encode a functional herpesvirus cleavage/packaging site;

(b) an amplicon plasmid comprising a sequence that encodes a functional herpesvirus cleavage/packaging site, a herpesvirus origin of DNA replication, and a sequence that encodes an immunomodulatory protein, a tumor-specific antigen, or an antigen of an infectious agent; and

(c) a nucleic acid sequence that encodes an accessory protein.

3. The method of claim 1 or claim 2, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

4. The method of claim 3, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

5. The method of claim 1 or claim 2, wherein the accessory protein inhibits the expression of a gene in the cell.

6. The method of claim 5, wherein the accessory protein is a virion host shutoff protein.

7. The method of claim 6, wherein the virion host shutoff protein is an HSV-1 virion host shutoff protein, an HSV-2 virion host shutoff protein, an HSV-3 virion host shutoff protein, bovine herpesvirus 1 virion host shutoff protein, bovine herpesvirus 1.1 virion host shutoff protein, gallid herpesvirus 1 virion host shutoff protein, gallid herpesvirus 2 virion host shutoff protein, suid herpesvirus 1 virion host shutoff protein, baboon herpesvirus 2 virion host shutoff protein, pseudorabies virus virion host shutoff protein, cercopithecine herpesvirus 7 virion host shutoff protein, meleagrid herpesvirus 1 virion host shutoff protein, equine herpesvirus 1 virion host shutoff protein, or equine herpesvirus 4 virion host shutoff protein.

8. The method of claim 6, wherein the virion host shutoff protein is operatively coupled to its native transcriptional control elements.

9. The method of claim 1 or claim 2, wherein the cell is further transfected with a sequence encoding a VP16 protein, wherein the VP16 protein is transiently or stably expressed.

10. The method of claim 9, wherein the VP16 protein is HSV1 VP16, HSV-2 VP16, bovine herpesvirus 1 VP16, bovine herpesvirus 1.1 VP16, gallid herpesvirus 1 VP16, gallid herpesvirus 2 VP16, meleagrid herpesvirus 1 VP16, or equine herpesvirus 4 VP16.

11. The method of claim 1 or claim 2, wherein the one or more packaging vectors comprises a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, a human artificial chromosome, or an F element plasmid.

12. The method of claim 1 or claim 2, wherein the one or more packaging vectors comprises a set of cosmids comprising cos6Δa, cos28, cos14, cos56, and cos48Δa.

13. The method of claim 1 or claim 2, wherein the one or more packaging vectors, individually or collectively, express the structural herpesvirus proteins.

14. The method of claim 1 or claim 2, wherein the herpesvirus origin of DNA replication is not present in the one or more packaging vectors.

15. The method of claim 1, wherein the amplicon plasmid further comprises a sequence encoding a therapeutic agent.

16. The method of claim 15, wherein the therapeutic agent is a protein or an RNA molecule.

17. The method of claim 16, wherein the RNA molecule is an antisense RNA molecule, RNAi, or a ribozyme.

18. The method of claim 16, wherein the protein is a receptor, a signaling molecule, a transcription factor, a growth factor, an apoptosis inhibitor, an apoptosis promoter, a DNA replication factor, an enzyme, a structural protein, a neural protein, or a histone.

19. The method of claim 16, wherein the protein is an immunomodulatory protein, a tumor-specific antigen, or an antigen of an infectious agent.

20. The method of claim 19, wherein the immunomodulatory protein is a cytokine or a costimulatory molecule.

21. The method of claim 20, wherein the cytokine is an interleukin, an interferon, or a chemokine.

22. The method of claim 20, wherein the costimulatory molecule is a B7 molecule or CD40L.

23. The method of claim 19, wherein the tumor-specific antigen is a prostate specific antigen.

24. The method of claim 19, wherein the infectious agent is a virus.

25. The method of claim 24, wherein the virus is a human immunodeficiency virus.

26. The method of claim 19, wherein the antigen of an infectious agent is gp120.

27. The method of claim 19, wherein the infectious agent is a bacterium or parasite.

28. The method of claim 2, wherein the immunomodulatory protein is a cytokine or a costimulatory molecule.

29. The method of claim 28, wherein the cytokine is an interleukin, an interferon, or a chemokine.

30. The method of claim 28, wherein the costimulatory molecule is a B7 molecule or CD40L.

31. The method of claim 2, wherein the tumor-specific antigen is a prostate specific antigen.

32. The method of claim 2, wherein the infectious agent is a virus.

33. The method of claim 32, wherein the virus is a human immunodeficiency virus.
34. The method of claim 2, wherein the antigen of an infectious agent is gp120.
35. The method of claim 2, wherein the infectious agent is a bacterium or parasite.
36. The method of claim 1 or claim 2, wherein the amplicon plasmid further comprises a promoter.
37. A cell transfected by the method of claim 1 or transduced by a herpesvirus amplicon particle made by the method of claim 1.
38. The cell of claim 37, wherein the cell is a neuron, a blood cell, a hepatocyte, a keratinocyte, a melanocyte, a neuron, a glial cell, an endocrine cell, an epithelial cell, a muscle cell, a prostate cell, or a testicular cell.
39. A cell transfected by the method of claim 2 or transduced by a herpesvirus amplicon particle made by the method of claim 2.
40. The cell of claim 39, wherein the cell is a neuron, a blood cell, a hepatocyte, a keratinocyte, a melanocyte, a neuron, a glial cell, an endocrine cell, an epithelial cell, a muscle cell, a prostate cell, or a testicular cell.
41. The cell of claim 39, wherein the cell is a malignant cell.
42. The cell of claim 39, wherein the cell is infected with an infectious agent.
43. The cell of claim 42, wherein the infectious agent is a virus, a bacterium, or a parasite.

44. The cell of claim 43, wherein the virus is an immunodeficiency virus.

45. A herpesvirus amplicon particle made by the method of claim 1.

46. The herpesvirus amplicon particle of claim 45, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

47. The herpesvirus amplicon particle of claim 46, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

48. The herpesvirus amplicon particle of claim 47, wherein the herpes simplex virus is a type 1 or a type 2 herpes simplex virus.

49. A herpesvirus amplicon particle made by the method of claim 2.

50. The herpesvirus amplicon particle of claim 49, wherein the herpesvirus is an alpha herpesvirus or an Epstein-Barr virus.

51. The herpesvirus amplicon particle of claim 50, wherein the alpha herpesvirus is a Varicella-Zoster virus, a pseudorabies virus, or a herpes simplex virus.

52. The herpesvirus amplicon particle of claim 51, wherein the herpes simplex virus is a type 1 or a type 2 herpes simplex virus.

53. A method of treating a patient who has cancer, or who may develop cancer, the method comprising administering to the patient an HSV amplicon particle of claim 19, wherein the protein is an immunomodulatory protein or a tumor-specific antigen, or an HSV amplicon particle made by the method of claim 2, wherein the protein is an immunomodulatory protein or a tumor-specific antigen.

54. A method of treating a patient who has cancer, or who may develop cancer, the method comprising administering to the patient the cell of claim 37, wherein the amplicon plasmid further encodes an immunomodulatory protein or a tumor-specific antigen, or the cell of claim tumor-specific antigen, or an HSV amplicon particle made by the method of claim 39, wherein the protein is an immunomodulatory protein or a tumor-specific antigen.

55. A method of treating a patient who has a disease caused by an infectious agent, or who may contract a disease caused by an infectious agent, the method comprising administering to the patient the herpesvirus amplicon particle of claim 45, wherein the amplicon plasmid further comprises a sequence that encodes an antigen of the infectious agent, or the cell of claim 39, wherein the amplicon plasmid comprises a sequence that encodes an antigen of an infectious agent.